Leaky Waveguide Devices as Simple Sensitive Optical Detectors for use in μTAS Applications.

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Abstract

Leaky Waveguide Devices (LWD) [1,2] are a class of optical waveguide where light is partially confined by reflection mechanisms other than total internal reflection (TIR). Since TIR is not used, the restriction that the waveguide must have a higher refractive index than the surrounding medium can be relaxed. It is then possible to provide lossy waveguiding in channels containing aqueous solutions. Since these reflection mechanisms always provide less than 100% reflectivity, light is gradually lost from the waveguide either by absorption or by radiative loss. Although this means that LWD cannot by used for long-distance light transmission, the typical propagation distances of a few millimetres to a few centimetres is sufficient for most μTAS sensing applications. A novel laminar-flow waveguide cytometer is described which uses Leaky Wave propagation to excite fluorescence.

1 Introduction

Previous work on using optical waveguide sensors as monitors of fluids in microchannels has concentrated on evanescent wave sensing using high index optical waveguides as one wall of the channel [2]. In the work described, Fresnel reflection is used as the light confinement mechanism. Fresnel reflection occurs at any interface between materials of different refractive index [3]. At high angles of incidence, the reflectivity can approach 100%, as shown in Figure 1. Unlike TIR, there is no evanescent field penetrating into the other material, since there is always some light transmitted. There is therefore no Goos-Hänchen shift on reflection, making exact analytical solutions for the mode indices possible. Since all LWD modes are lossy, light will leak from the waveguide. This light is not lost in random directions, however, but couples out at well-defined angles where the propagation constants of the waveguide mode and the substrate match. The same process can couple light into the LWD mode by illumination of the substrate/waveguide interface at the correct angle. Prism coupling can therefore be used to couple light in and out of the waveguide layer. The structure of the LWD can be very simple. No optical coatings are required, since it is merely the difference in refractive index between the channel walls and the channel contents which provides the reflectivity.

Previous work [4] had concentrated on the use of Anti-Resonant Reflecting Optical Waveguides (ARROW) as a method of providing optical confinement in liquid-filled channels. These devices provide better confinement than Leaky Wave devices, but require thin-film coatings, and thus cannot easily be fabricated on polymer substrates. For this reason, simple Leaky Wave devices were used with polymer substrates.

The original aim of the research was to miniaturise bioanalytical devices ranging from flow cytometers to immunosensors. The objective was to produce good quality, easily reproducible and inexpensive disposable devices. Two different fabrication methods were developed. Firstly, dry film negative photoresist was laminated onto polymer substrates (between 0.75 and 2 mm thick) with a hot roll laminator. Multiple chip photomasks were then used to create the channel pattern by UV exposure. The exposed sheet was developed in a 1% potassium carbonate solution to remove the non-crosslinked resist. Finally, an unlaminated polycarbonate sheet with predrilled access holes was thermally bonded onto the patterned chip. This fabrication method can be used to produce multilayer 3-D channel networks for μ-TAS applications. We have produced a five-layer 3-D channel networks using this method.

In the second method, polystyrene injection-moulded parts were fabricated using conventionally machined brass tooling. Feature sizes of ~100 μm can be created in this way. In addition, coupling prisms and lenses can be fabricated as part of the device.

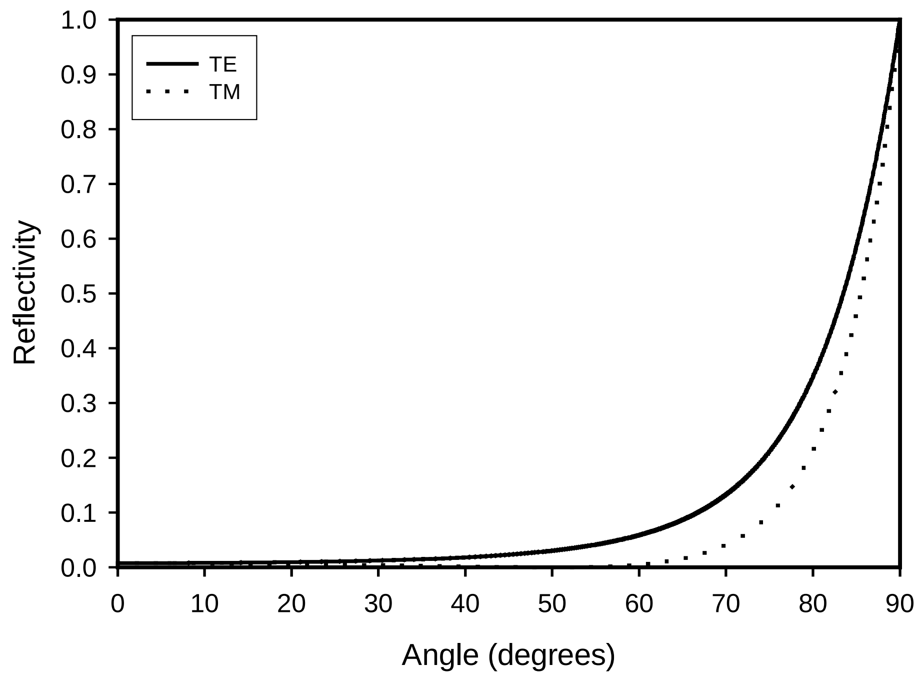

Figure 1. Fresnel reflection at a water/polystyrene interface.

In either case, analyte solution was pumped along the microfabricated channels. These channels thus formed a low refractive index region sandwiched between higher index substrates. A refractive index profile of this sort cannot support conventional optical modes. However, leaky modes can be supported by such low-index waveguides as a result of the high Fresnel reflections at glancing incidence at the sample-substrate interfaces. These LW modes have the property that their optical leakage is very low, which means that they are able to propagate several centimeters without suffering significant loss.

2 Theory of Symmetric LWD

We can determine the modes of a Leaky Waveguide by using a simple model which consists of a plane waveguiding layer of thickness h and refractive index n_w bounded on either side by semi-infinite (much thicker than the wavelength) substrate and superstrate layers of index n_s. Figure 1 shows this structure diagramatically, with the addition of a coupling prism of index n_s. The mode equation for this structure can be written as:

$$\Phi_{tot} = 2\Phi_z + 2\Phi_{w,s} = 2m\pi \qquad (1)$$

Where Φ_{tot} is the total phase shift for one complete back-and-forth reflection between the two waveguide/substrate boundaries, Φ_z is the phase shift for the propagation of the wave from one boundary to the other and $\Phi_{w,s}$ is the phase shift on reflection at the waveguide/substrate boundary. To satisfy the transverse resonance condition, Φ_{tot} must be an integral multiple of 2π.

We can show that:

$$\Phi_Z = \frac{2\pi}{\lambda}\left(\sqrt{n_w^2 - N^2}\right)$$

$$\Phi_{w,s} = \pi \qquad \text{for TE modes and TM modes where } \theta_{\mathrm{i}} > \theta_p$$

$$= 0 \qquad \text{for TM modes where } \theta_{\mathrm{i}} < \theta_p$$

Where N is the real part of the complex mode index β, λ is the vacuum wavelength, m is the mode number, θ_i the angle of incidence on the waveguide/substrate boundary and θ_p the polarisation angle. Making the appropriate substitutions into (1) gives:

$$\frac{4\pi h}{\lambda}\left(\sqrt{n_w^2 - N^2}\right) = 2m\pi \qquad (2)$$

For TE and TM modes where $\theta_i > \theta_p$ or

$$\frac{4\pi h}{\lambda}\left(\sqrt{n_w^2 - N^2}\right) + 2\pi = 2m\pi \qquad (3)$$

For TM modes where $\theta_i < \theta_p$.

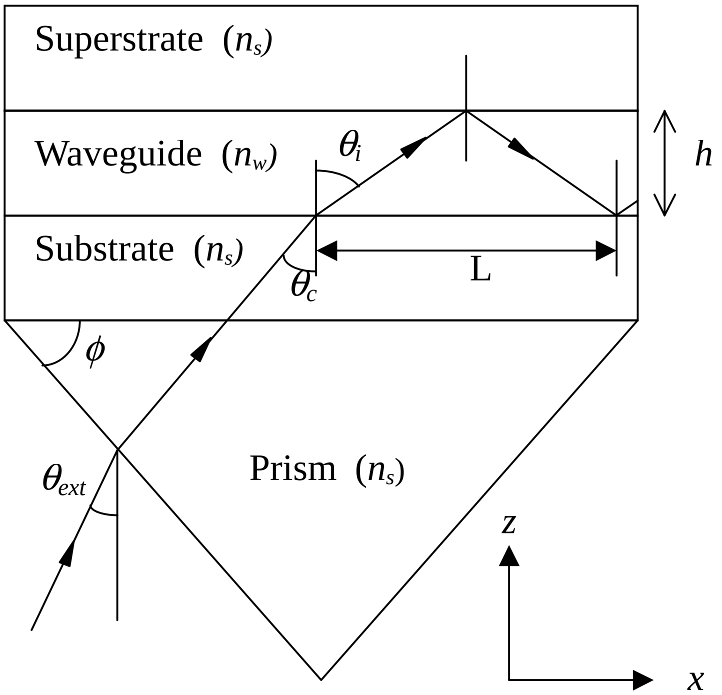

Figure 2. Symmetric LWD block diagram

Since the 2π term in (2) is constant, it can be removed, giving a single mode equation for all TE and TM modes:

$$\frac{4\pi h}{\lambda}\left(\sqrt{n_w^2 - N^2}\right) = 2m\pi \tag{4}$$

This can be rearranged to give N:

$$N = \sqrt{n_w^2 - \left(\frac{m\lambda}{2h}\right)^2} \tag{5}$$

The imaginary part K of the complex mode index can be calculated from the reflectivity coefficients for TE and TM polarisations and the "hopping length" L, the distance travelled in the x direction between successive reflections at one of the boundaries. Since there is no Goos-Hänchen shift associated with Fresnel reflection, L is simply given by the geometrical distance:

$$L = 2h\tan\theta_i \tag{6}$$

The reflectivity coefficient is given by:

$$R = \left(\frac{m\lambda n_s^{2p} - 2hn_w^{2p}\sqrt{n_s^2 - N^2}}{m\lambda n_s^{2p} + 2hn_w^{2p}\sqrt{n_s^2 - N^2}}\right)^2 \tag{7}$$

Where $p = 0$ for TE and $p = 1$ for TM modes. The attenuation factor α links the imaginary part K of the complex mode index to the loss per unit length:

$$I_x = I_0 e^{-\alpha x} \quad \text{or} \quad \ln\frac{I_x}{I_0} = -\alpha x \tag{8}$$

Where

$$\alpha = \frac{4\pi K}{\lambda} \tag{9}$$

Over the hopping distance L, the light undergoes reflections at the waveguide-substrate and waveguide-superstrate interfaces, and is thus reduced to R^2 of its original intensity since the reflectivity at both boundaries is the same. Substituting R^2 for I_x/I_0, and for α we obtain:

$$\ln R^2 = 2\ln R = -\frac{4\pi KL}{\lambda} = -\frac{8\pi Kh\tan\theta_i}{\lambda} \tag{10}$$

Since

$$\sin\theta_i = \frac{N}{n_w}$$

then

$$\tan\theta_i = \frac{N}{\sqrt{n_w^2 - N^2}} \tag{11}$$

Substituting for $\tan\theta_i$ and rearranging (10), we obtain the expression for K:

$$K = -\frac{\lambda^2 m \ln R}{8\pi h^2 N} \tag{12}$$

We can use equations (5) and (12) to derive some general design rules for LWD devices. Firstly, as the thickness, h, of the leaky waveguide increases, the number of modes increases and the loss for both TE and TM modes decreases. Secondly, the losses are different for TE and TM modes having the same mode number; the TM mode will always have higher losses. Thirdly, the losses for a given geometry will be reduced as the difference in refractive index between the waveguide and substrate is increased.

The imaginary part of the mode index determines how far a mode will propagate in the waveguide. It also determines the coupling length over which light will be coupled into the waveguide. If the imaginary part is too small, the coupling length will be very long, and a finite input beam width will result in a low intensity within the waveguide. The imaginary part must be tailored to produce the appropriate coupling length to maximise the intensity within the waveguide.

3 Results

The channels that we have fabricated are approximately 100 µm wide and 30 µm deep. These dimensions are ideal for the passage of yeast cells. Using LW optical propagation within the channels, we have demonstrated the detection of single Green Fluorescent Protein (GFP) containing cells in parallel detection microfabricated chips. In addition, the amount of scattering from the cells can also be monitored. Thus we are able to determine the size of individual yeast cells, and also the concentration of GFP within the cells.

To test the utility of Leaky Wave excitation for monitoring fluorescence, a simple channel 500 µm wide by 30 µm deep was constructed using polycarbonate substrates and dry-film photoresist. The channel was filled with a dilute fluorescein solution and the first Leaky Wave mode excited using a 473 nm laser via an equilateral coupling prism. A 5000 pixel linear CCD was used to monitor the decay of fluorescence along the channel. A 1:1 imaging lens was used to form an image of the channel on the CCD. Figure 3 shows an approximately exponential decay of fluorescence along the channel. Given that the CCD pixel pitch is 7 µm, it can be seen that the decay length is approximately 6.3 mm.

Multiple parallel fluid streams are fabricated in order to increase the throughput of the cells. Conventionally, these are produced by fabricating individual flow channels on a polymer or glass substrate. The channels may be separated from adjacent ones by strips of photoresist or by ridges of polymer produced by injection moulding. Figure 4 shows a ten channel flow system produced using dry-film photoresist on polycarbonate sheet substrates. The LW modes are launched along the channels through a glass equilateral prism using an expanded beam of sufficient width to cover all ten channels. An index-matching fluid of the correct index was used to couple optically the prism and polymer flow-channel device. The bright dots within the channel are individual 5µm fluorescent beads excited at 473 nm.

While such flow channels work adequately, it would be desirable to remove the need to use photoresist or polymer to separate adjacent channels. The reasons for this are that the fabrication process is difficult, especially if a large number of flow channels need to be produced, and there are refractive index steps in the direction perpendicular to the flow channels. This makes it difficult to launch an optical mode orthogonally across all the channels to excite fluorescence, and causes extra reflection losses at each interface unless the separating material has the same index as the channel contents.

To overcome these drawbacks of conventional multiple flow channels, we have exploited laminar flow microfabricated structures. The fabrication process for such a device is simpler than that for conventional microfabricated systems since the need to physically separate adjacent flow channels is eliminated.

A diluent, such as phosphate-buffered saline and the cell suspension are directed by air pressure or syringe pump into the chip. The diluent streams are formed when they flow around separators which are formed from photoresist. The cell suspension is injected behind these separators through drilled access holes with a diameter of approximately 150 µm. The pressure of the diluent fluid against the cell streams aligns the particles in a *single file* and allows precise centring of these particle streams. The sample streams travel by laminar flow along the chip. The structure of the chip lends itself to optical interrogation by LW modes. This means that each cell can be interrogated *individually* by the laser light. Since there are no longer any material boundaries between the individual cell streams, a leaky wave mode can be launched orthogonally across all the channels. Suitable detectors can then be arranged above or below the device to collect fluorescence or scattered light from the particles as they intercept the orthogonal Leaky Wave mode.

Because of the ease of fabrication of laminar flow devices, it would be possible to produce a chip with many hundreds of sample streams. We have realised a

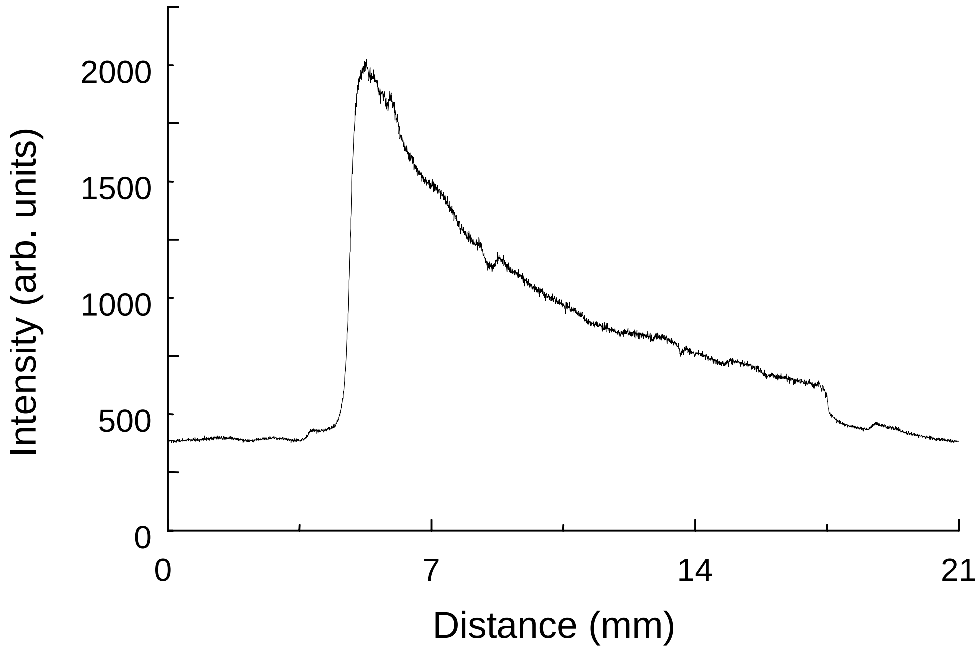

Figure 3. Decay of Leaky Wave mode monitored by fluorescence emission excited at 473 nm.

ten channel version to match currently-available avalanche photodiode arrays for the detection of the scattered and the fluorescent light of the cells. Figure 5 shows a ten channel laminar flow system where the LW mode is propagating perpendicular to the flow streams. Ten individual spots of fluorescence emission are visible where the LW mode intersects the fluorescein-containing flow stream. Such laminar flow behaviour persists along the entire length of the flow channel, some 2 cm in total. Figure 6 shows the propagation of light along the length of each flow stream, showing that each stream remains discrete until the end of the channel. Finally, Figure 7 shows the output from each element of a ten channel avalanche photodiode array monitoring fluorescence from 5 µm labelled beads flowing in the laminar system. Each trace shows a four second segment of data obtained from this system.

Conclusions

Theoretical analysis of Leaky Wave modes confined by Fresnel reflection has shown that these modes can be launched by simple prism coupling into polymer fabricated channels containing materials of lower refractive index than the substrate. Experimental confirmation of the existence of these modes has been obtained by monitoring the decay of fluorescence along such a channel.

We have also shown that these modes can be used to monitor fluorescent species in discrete channel arrays by using an expanded beam and coupling parallel to the channels. This reduces the intensity in each channel, so an alternative approach using the laminar flow behaviour of microfluidic systems was developed. In this system, the individual flow streams were separated by flow streams of a buffer solution containing no fluorescent species. Laminar flow behaviour aver distances of up to 2 cm was observed, and orthogonal Leaky Wave propagation observed.

References

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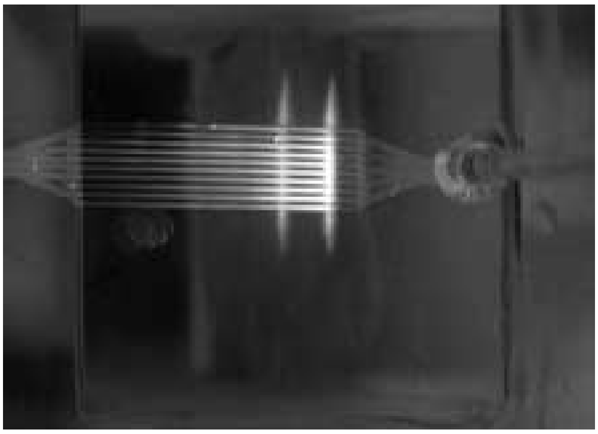

Figure 4. Ten channel flow system using parallel Leaky Wave excitation at 473 nm.

Figure 5. Ten channel laminar flow system using orthogonal Leaky Wave excitation of fluorescence at 473 nm

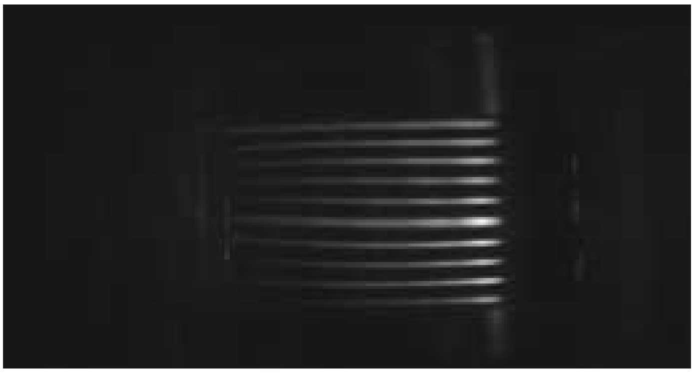

Figure 6. Ten channel laminar flow system using parallel Leaky Wave excitation at 473 nm.

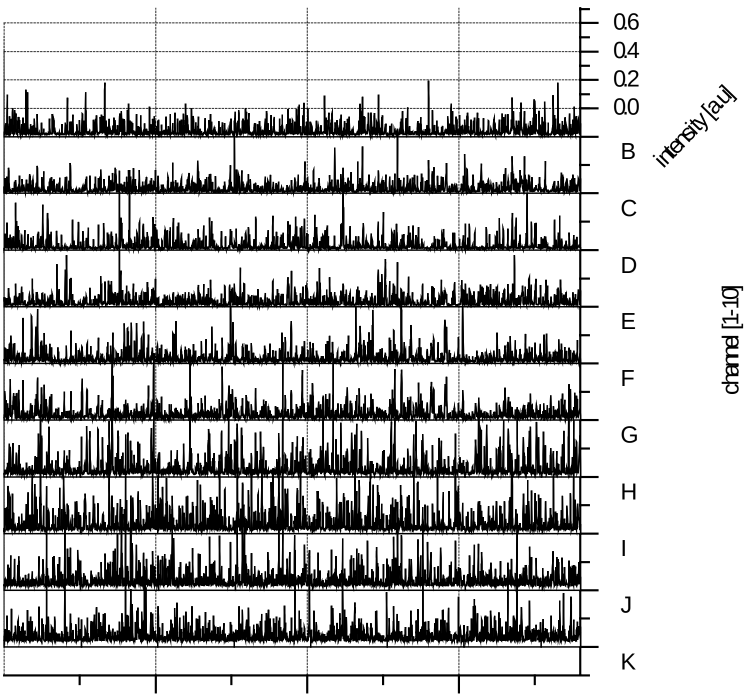

Figure 7. Data traces obtained from ten channel avalanche photodiode array using 10 channel orthogonal Leaky Wave excited laminar flow system.